**Specific Aims**

With the innate goal in organisms to pass their genetic information from generation to generation, it becomes easy to see the importance of keeping DNA unchanged and intact. To ensure this consistency, our body possesses numerous mechanisms to detect and repair DNA damage1. An immense number of proteins play a role in the repair of damaged DNA, one such protein being nibrin (NBN). Mutated NBN genes have been known to cause the autosomal recessive disease Nijmegen Breakage Syndrome (NBS). NBS is an extremely rare genetic condition characterized by small head size, distinctive facial features, intellectual disability, short stature, immunodeficiency, and many, many additional symptoms. The most relevant to the lives of these individuals, however, is their increased predisposition to cancer, affecting around 50 percent before the age of 15 and causing the vast majority of patient deaths2-5.

Recent research has shown the disease also influences heterozygotes, suggesting that one healthy NBN gene is not sufficient6. Specifically, carriers experience higher spontaneous and induced chromosome instability and predisposition to malignancies7. *However, the reason as to why patients with different mutations may express the same clinical phenotype and patients with the same mutations may have different phenotypes remains unclear.* Although those affected by NBS, and any other recessive mutation, may be rare, heterozygous carriers are quiet prevalent and therefore have a noticeable contribution to our gene pool6. Identification of individuals heterozygous for NBS could aid in genetic counseling and future awareness surrounding situations in which DNA damage may result8.

**Here, we will test the hypothesis that differences in mutations of the NBN protein affect the level of chromosomal instability and therefore vary in phenotypic outcomes.** Our hypothesis has been formulated on the basis of published data presenting case studies of several NBS carriers and the fragility of their chromosomes.

Our ***long-term goal***is to understand the various mutations of NBN and their influence on those heterozygous for Nijmegen Breakage Syndrome. We will then incorporate the findings from these studies and attempt to begin tackling their relation, if any, to those homozygous for NBS. The ***objective*** of this proposal is use this knowledge gained to better accommodate medical assistance and treatment opportunities for all those affected by NBS. Focusing on heterozygous carriers of NBS, we will pursue the following three specific aims:

***Aim 1. Establish any consistency in hereditability between NBN mutations and phenotypes.*** Preform case studies on various familial lines to determine if NBN mutations remain consistent in both site of mutation and level of chromosome instability.

***Aim 2. Determine if the amount of nibrin in cells has correlation with various phenotypes.*** Extract NBN protein from various patients for western blot analysis. Compare protein content with phenotypic severity.

***Aim 3. Determine if there lies a correlation between site-specific truncations of the NBN protein and various phenotypes.*** Use crisper/cas9 to induce site-specific mutations in the NBN gene. Compare the stability of the chromosomes with the various sized proteins.